

\* \* \* \* \* STN Columbus \* \* \* \* \*

FILE 'HOME' ENTERED AT 16:37:59 ON 09 JAN 2008

=> fil .bec

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
0.21	0.21

FULL ESTIMATED COST

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIODBASE, BIOTECHNO, WPIDS' ENTERED AT 16:38:14 ON 09 JAN 2008  
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11 FILES IN THE FILE LIST

=> s chaperon? or aggregat?(5a) (inhibit? or suppress? or prevent? or decreas?)  
FILE 'MEDLINE'

	18469	CHAPERON?
	130845	AGGREGAT?
	1390221	INHIBIT?
	324425	SUPPRESS?
	1132552	PREVENT?
	1134113	DECREAS?
	28768	AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L1	46384	CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)

FILE 'SCISEARCH'

	19109	CHAPERON?
	169663	AGGREGAT?
	1177864	INHIBIT?
	352434	SUPPRESS?
	496182	PREVENT?
	1198877	DECREAS?
	14577	AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L2	32757	CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)

FILE 'LIFESCI'

	6502	CHAPERON?
	33673	AGGREGAT?
	387405	INHIBIT?
	106555	SUPPRESS?
	108220	PREVENT?
	279513	DECREAS?
	3067	AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L3	9211	CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)

FILE 'BIOTECHDS'

	872	CHAPERON?
	4794	AGGREGAT?
	67214	INHIBIT?
	12229	SUPPRESS?
	33717	PREVENT?
	30017	DECREAS?
	719	AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L4	1555	CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)

FILE 'BIOSIS'

	15922	CHAPERON?
	142346	AGGREGAT?
	1582074	INHIBIT?
	351286	SUPPRESS?

550985 PREVENT?  
1354548 DECREAS?  
18518 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L5 33580 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'EMBASE'

13870 CHAPERON?  
108677 AGGREGAT?  
1279902 INHIBIT?  
299727 SUPPRESS?  
878779 PREVENT?  
1056443 DECREAS?  
18016 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L6 31123 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'HCAPLUS'

18675 CHAPERON?  
250264 AGGREGAT?  
1993587 INHIBIT?  
440923 SUPPRESS?  
996586 PREVENT?  
2453976 DECREAS?  
34414 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L7 52083 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'NTIS'

117 CHAPERON?  
13507 AGGREGAT?  
22511 INHIBIT?  
15448 SUPPRESS?  
54431 PREVENT?  
54034 DECREAS?  
172 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L8 286 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'ESBIOBASE'

11228 CHAPERON?  
44845 AGGREGAT?  
542878 INHIBIT?  
146577 SUPPRESS?  
180476 PREVENT?  
449169 DECREAS?  
5712 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L9 16269 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'BIOTECHNO'

5718 CHAPERON?  
22679 AGGREGAT?  
301415 INHIBIT?  
79558 SUPPRESS?  
71195 PREVENT?  
171676 DECREAS?  
3551 AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L10 8911 CHAPERON? OR AGGREGAT? (5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

FILE 'WPIDS'

643 CHAPERON?  
68414 AGGREGAT?  
286804 INHIBIT?

266194 SUPPRESS?  
1903331 PREVENT?  
286510 DECREAS?  
8142 AGGREGAT?(5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)  
L11 8754 CHAPERON? OR AGGREGAT?(5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

TOTAL FOR ALL FILES

L12 240913 CHAPERON? OR AGGREGAT?(5A) (INHIBIT? OR SUPPRESS? OR PREVENT? OR  
DECREAS?)

=> s l12(10a) fusion

FILE 'MEDLINE'

153107 FUSION

L13 109 L1 (10A) FUSION

FILE 'SCISEARCH'

139607 FUSION

L14 109 L2 (10A) FUSION

FILE 'LIFESCI'

43845 FUSION

L15 72 L3 (10A) FUSION

FILE 'BIOTECHDS'

27435 FUSION

L16 70 L4 (10A) FUSION

FILE 'BIOSIS'

111256 FUSION

L17 114 L5 (10A) FUSION

FILE 'EMBASE'

89782 FUSION

L18 97 L6 (10A) FUSION

FILE 'HCAPLUS'

280236 FUSION

L19 292 L7 (10A) FUSION

FILE 'NTIS'

23224 FUSION

L20 0 L8 (10A) FUSION

FILE 'ESBIOBASE'

47019 FUSION

L21 89 L9 (10A) FUSION

FILE 'BIOTECHNO'

42345 FUSION

L22 61 L10(10A) FUSION

FILE 'WPIDS'

56908 FUSION

L23 73 L11(10A) FUSION

TOTAL FOR ALL FILES

L24 1086 L12(10A) FUSION

=> s l12(10a) (archae? or methanococcus or thermococcus or methanosarcina)

FILE 'MEDLINE'

13255 ARCHAE?

1058 METHANOCOCCUS

451 THERMOCOCCUS

785 METHANOSARCINA  
L25 132 L1 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'SCISEARCH'

23833 ARCHAE?  
1391 METHANOCOCCUS  
671 THERMOCOCCUS  
1449 METHANOSARCINA  
L26 158 L2 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'LIFESCI'

8345 ARCHAE?  
829 METHANOCOCCUS  
346 THERMOCOCCUS  
849 METHANOSARCINA  
L27 93 L3 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'BIOTECHDS'

1773 ARCHAE?  
241 METHANOCOCCUS  
245 THERMOCOCCUS  
426 METHANOSARCINA  
L28 31 L4 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'BIOSIS'

31751 ARCHAE?  
1632 METHANOCOCCUS  
632 THERMOCOCCUS  
1614 METHANOSARCINA  
L29 145 L5 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'EMBASE'

9952 ARCHAE?  
1017 METHANOCOCCUS  
415 THERMOCOCCUS  
948 METHANOSARCINA  
L30 116 L6 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'HCAPLUS'

27262 ARCHAE?  
1612 METHANOCOCCUS  
826 THERMOCOCCUS  
1608 METHANOSARCINA  
L31 228 L7 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'NTIS'

4801 ARCHAE?  
37 METHANOCOCCUS  
3 THERMOCOCCUS  
49 METHANOSARCINA  
L32 4 L8 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'ESBIOBASE'

10844 ARCHAE?  
674 METHANOCOCCUS  
375 THERMOCOCCUS  
575 METHANOSARCINA

L33 118 L9 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'BIOTECHNO'

5361 ARCHAE?

719 METHANOCOCCUS

284 THERMOCOCCUS

662 METHANOSARCINA

L34 78 L10 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

FILE 'WPIDS'

707 ARCHAE?

108 METHANOCOCCUS

162 THERMOCOCCUS

87 METHANOSARCINA

L35 23 L11 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

TOTAL FOR ALL FILES

L36 1126 L12 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC  
INA)

=> s l24 and l36

FILE 'MEDLINE'

L37 1 L13 AND L25

FILE 'SCISEARCH'

L38 1 L14 AND L26

FILE 'LIFESCI'

L39 0 L15 AND L27

FILE 'BIOTECHDS'

L40 4 L16 AND L28

FILE 'BIOSIS'

L41 1 L17 AND L29

FILE 'EMBASE'

L42 1 L18 AND L30

FILE 'HCAPLUS'

L43 14 L19 AND L31

FILE 'NTIS'

L44 0 L20 AND L32

FILE 'ESBIOBASE'

L45 1 L21 AND L33

FILE 'BIOTECHNO'

L46 0 L22 AND L34

FILE 'WPIDS'

L47 4 L23 AND L35

TOTAL FOR ALL FILES

L48 27 L24 AND L36

=> s peptidyl prolyl(3w)isomerase# or ppiase# or ppi

FILE 'MEDLINE'

14055 PEPTIDYL

6254 PROLYL

888 PEPTIDYL PROLYL

(PEPTIDYL (W) PROLYL)  
15290 ISOMERASE#  
820 PEPTIDYL PROLYL (3W) ISOMERASE#  
391 PPIASE#  
4179 PPI  
L49 5076 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'SCISEARCH'

4536 PEPTIDYL  
5921 PROLYL  
1401 PEPTIDYL PROLYL  
(PEPTIDYL (W) PROLYL)  
12681 ISOMERASE#  
1340 PEPTIDYL PROLYL (3W) ISOMERASE#  
395 PPIASE#  
4586 PPI  
L50 6003 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'LIFESCI'

2234 "PEPTIDYL"  
1708 "PROLYL"  
482 PEPTIDYL PROLYL  
("PEPTIDYL" (W) "PROLYL")  
4527 ISOMERASE#  
458 PEPTIDYL PROLYL (3W) ISOMERASE#  
208 PPIASE#  
798 PPI  
L51 1289 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOTECHDS'

291 PEPTIDYL  
344 PROLYL  
82 PEPTIDYL PROLYL  
(PEPTIDYL (W) PROLYL)  
2476 ISOMERASE#  
78 PEPTIDYL PROLYL (3W) ISOMERASE#  
36 PPIASE#  
184 PPI  
L52 269 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOSIS'

5328 PEPTIDYL  
6487 PROLYL  
1069 PEPTIDYL PROLYL  
(PEPTIDYL (W) PROLYL)  
15328 ISOMERASE#  
996 PEPTIDYL PROLYL (3W) ISOMERASE#  
423 PPIASE#  
5528 PPI  
L53 6612 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'EMBASE'

3390 "PEPTIDYL"  
4499 "PROLYL"  
807 PEPTIDYL PROLYL  
("PEPTIDYL" (W) "PROLYL")  
8932 ISOMERASE#  
749 PEPTIDYL PROLYL (3W) ISOMERASE#  
318 PPIASE#  
3251 PPI  
L54 4057 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'HCAPLUS'

6944 PEPTIDYL  
8879 PROLYL

1412 PEPTIDYL PROLYL  
 (PEPTIDYL (W) PROLYL)  
 20028 ISOMERASE#  
 1331 PEPTIDYL PROLYL (3W) ISOMERASE#  
 580 PPIASE#  
 4381 PPI  
 L55 5828 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'NTIS'

31 PEPTIDYL  
 21 PROLYL  
 4 PEPTIDYL PROLYL  
 (PEPTIDYL (W) PROLYL)  
 104 ISOMERASE#  
 3 PEPTIDYL PROLYL (3W) ISOMERASE#  
 1 PPIASE#  
 270 PPI  
 L56 273 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'ESBIOBASE'

2046 PEPTIDYL  
 2213 PROLYL  
 647 PEPTIDYL PROLYL  
 (PEPTIDYL (W) PROLYL)  
 5695 ISOMERASE#  
 602 PEPTIDYL PROLYL (3W) ISOMERASE#  
 279 PPIASE#  
 1558 PPI  
 L57 2211 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOTECHNO'

1406 PEPTIDYL  
 1423 PROLYL  
 412 PEPTIDYL PROLYL  
 (PEPTIDYL (W) PROLYL)  
 4091 ISOMERASE#  
 386 PEPTIDYL PROLYL (3W) ISOMERASE#  
 171 PPIASE#  
 315 PPI  
 L58 731 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

FILE 'WPIDS'

981 PEPTIDYL  
 1223 PROLYL  
 143 PEPTIDYL PROLYL  
 (PEPTIDYL (W) PROLYL)  
 2153 ISOMERASE#  
 141 PEPTIDYL PROLYL (3W) ISOMERASE#  
 52 PPIASE#  
 706 PPI  
 L59 867 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

TOTAL FOR ALL FILES

L60 33216 PEPTIDYL PROLYL (3W) ISOMERASE# OR PPIASE# OR PPI

=> s 124 and 160

FILE 'MEDLINE'

L61 1 L13 AND L49

FILE 'SCISEARCH'

L62 1 L14 AND L50

FILE 'LIFESCI'

L63 2 L15 AND L51

FILE 'BIOTECHDS'  
L64 9 L16 AND L52

FILE 'BIOSIS'  
L65 2 L17 AND L53

FILE 'EMBASE'  
L66 1 L18 AND L54

FILE 'HCAPLUS'  
L67 15 L19 AND L55

FILE 'NTIS'  
L68 0 L20 AND L56

FILE 'ESBIOBASE'  
L69 2 L21 AND L57

FILE 'BIOTECHNO'  
L70 0 L22 AND L58

FILE 'WPIDS'  
L71 8 L23 AND L59

TOTAL FOR ALL FILES  
L72 41 L24 AND L60

=> s (l48 or l72) not 2004-2008/py

FILE 'MEDLINE'  
2589167 2004-2008/PY  
(20040000-20089999/PY)  
L73 0 (L37 OR L61) NOT 2004-2008/PY

FILE 'SCISEARCH'  
4718144 2004-2008/PY  
(20040000-20089999/PY)  
L74 0 (L38 OR L62) NOT 2004-2008/PY

FILE 'LIFESCI'  
536487 2004-2008/PY  
L75 0 (L39 OR L63) NOT 2004-2008/PY

FILE 'BIOTECHDS'  
107093 2004-2008/PY  
L76 2 (L40 OR L64) NOT 2004-2008/PY

FILE 'BIOSIS'  
2190807 2004-2008/PY  
L77 0 (L41 OR L65) NOT 2004-2008/PY

FILE 'EMBASE'  
2266271 2004-2008/PY  
L78 0 (L42 OR L66) NOT 2004-2008/PY

FILE 'HCAPLUS'  
5177725 2004-2008/PY  
L79 3 (L43 OR L67) NOT 2004-2008/PY

FILE 'NTIS'  
63115 2004-2008/PY  
L80 0 (L44 OR L68) NOT 2004-2008/PY

FILE 'ESBIOBASE'  
1321869 2004-2008/PY  
L81 0 (L45 OR L69) NOT 2004-2008/PY



FILE 'BIOTECHNO'

586 2004-2008/PY

L82 0 (L46 OR L70) NOT 2004-2008/PY

FILE 'WPIDS'

4380422 2004-2008/PY

L83 0 (L47 OR L71) NOT 2004-2008/PY

TOTAL FOR ALL FILES

L84 5 (L48 OR L72) NOT 2004-2008/PY

=> dup rem l84

PROCESSING COMPLETED FOR L84

L85 5 DUP REM L84 (0 DUPLICATES REMOVED)

=> d tot

L85 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
TI New recombinant DNA molecule encoding a fusion protein comprising a  
sequence coding for a target polypeptide of a sequence coding for an FKBP  
chaperone, useful for immunizing animals or in producing a vaccine;  
vector-mediated recombinant protein gene transfer and expression in  
host cell for use in recombinant vaccine preparation  
AU SCHOLZ C; ANDRES H; FAATZ E; ENGEL A; SCHMITT U; BAZARSUREN A;  
SCHAARSCHMIDT P  
AN 2003-11133 BIOTECHDS  
PI WO 2003000878 3 Jan 2003 *equiv. to US 2003/0096352*

L85 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
TI Producing a soluble complex comprising an essentially insoluble target  
protein and a peptidyl-prolyl-isomerase  
class chaperone, useful in detecting antibodies to HIV, comprises  
solubilizing protein and the chaperone in a buffer;  
plasmid-mediated recombinant fusion protein gene transfer and  
expression in host cell for use in HIV virus diagnosis and recombinant  
vaccine  
AU SCHOLZ C; ANDRES H; FAATZ E; ENGEL A; SIZMANN D  
AN 2003-11659 BIOTECHDS  
PI WO 2003000877 3 Jan 2003 *equiv. to 2003/0176665*

L85 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN  
TI Recombinant protein expression as chaperonin fusion  
protein for X-ray crystal structure analysis  
SO Jpn. Kokai Tokkyo Koho, 8 pp.  
CODEN: JKXXAF

IN Furuya, Masahiro; Hata, Junichi  
AN 2003:734803 HCAPLUS  
DN 139:257016

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003261597	A	20030919	JP 2002-353990	20021205

L85 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN  
TI Method for refolding molecules of polypeptides containing Ig domains  
SO PCT Int. Appl., 35 pp.  
CODEN: PIXXD2  
IN Fersht, Alan Roy; Altamirano, Myriam Marlenne; Woolfson, Adrian; Milstein,  
Cesar  
AN 2000:666754 HCAPLUS  
DN 133:251276

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000055183	A1	20000921	WO 2000-GB987	20000316

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,

CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,  
 IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,  
 MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,  
 SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW,  
 AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,  
 DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,  
 CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  
 CA 2364568 A1 20000921 CA 2000-2364568 20000316  
 EP 1161440 A1 20011212 EP 2000-911054 20000316  
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, SI, LT, LV, FI, RO

L85 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN  
 TI The chaperonin cycle cannot substitute for prolyl isomerase activity, but  
 GroEL alone promotes productive folding of a cyclophilin-sensitive  
 substrate to a cyclophilin-resistant form  
 SO EMBO Journal (1997), 16(15), 4568-4578  
 CODEN: EMJODG; ISSN: 0261-4189  
 AU Von Ahsen, Oliver; Tropschug, Maximilian; Pfanner, Nikolaus; Rassow,  
 Joachim  
 AN 1997:544620 HCAPLUS  
 DN 127:244366

=> d ab 1-5

L85 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
 AB DERWENT ABSTRACT:  
 NOVELTY - A recombinant DNA molecule encoding a fusion protein,  
 comprising at least one nucleotide sequence coding for a target  
 polypeptide and upstream to at least one nucleotide sequence coding for a  
 FKBP chaperone consisting of FkpA, SlyD or trigger factor, is new.  
 DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
 following: (1) an expression vector comprising operably linked a  
 recombinant DNA molecule above; (2) a host cell transformed with the  
 expression vector; (3) producing a fusion protein by culturing host cells  
 of (2), expressing the fusion protein, and purifying the fusion protein;  
 (4) a recombinantly produced fusion protein comprising at least one  
 polypeptide sequence corresponding to a FKBP chaperone selected from of  
 FkpA, SlyD and trigger factor, and at least one polypeptide sequence  
 corresponding to a target peptide, and at least one peptidic linker  
 sequence of 10-100 amino acids; and (5) a composition comprising a  
 recombinantly produced fusion protein of (4), and a pharmaceutical  
 excipient.  
 BIOTECHNOLOGY - Preferred DNA: The recombinant DNA molecule further  
 comprises at least one nucleotide sequence coding for a peptidic linker  
 of 10-100 amino acids located in between the sequence coding for the  
 target polypeptide and the sequence coding for the FKBP chaperone. The  
 recombinant DNA molecule comprises 1 or 2 nucleotide sequence coding for  
 the FKBP chaperone. The 2 sequences coding for a FKBP chaperone are  
 located upstream of the sequence coding for the target polypeptide, where  
 one sequence coding for a PPI chaperone is located upstream of  
 the target polypeptide and the other sequence coding for a PPI  
 chaperone is located downstream of the sequence coding for the target  
 peptide. The recombinant DNA molecule may also comprise 2 nucleic acid  
 sequences coding for a linker polypeptide of 10-100 amino acids, where  
 the 2 nucleic acid sequences coding for a linker of 10-100 amino acids  
 are different or at least one of the linker sequences codes for a  
 polypeptide linker comprising a proteolytic cleavage site. Preferred  
 Fusion Protein: The fusion protein comprises 1 or 2 polypeptide sequences  
 corresponding to the FKBP chaperone, where one of the two FKBP chaperones  
 is located N-terminal and the other FKBP chaperone is located C-terminal  
 to the target polypeptide. The peptidic linker sequences comprise a  
 proteolytic cleavage site, and the target protein comprises a polypeptide

from an infectious organism. The polypeptide comprises at least one diagnostically relevant epitope of an infectious organism.

ACTIVITY - Immunostimulant. No supporting data provided.

MECHANISM OF ACTION - Vaccine.

USE - The recombinantly produced fusion protein is useful in providing an efficient expression system for recombinant proteins, for immunization of laboratory animals, in the production of a vaccine, or in an immunoassay (claimed).

EXAMPLE - The restriction site BamHI in the coding region of the mature E. coli FkpA was deleted using the QuikChange site-directed mutagenesis kit. HIV-1 gp41 (535-681)-His6 was cloned and expressed in a T7 promoter-based expression system. The gene fragment encoding amino acids 535-681 from HIV-1 envelope protein was PCR amplified from the T7-based expression vector, and was inserted into EckFpA(DELTA BamHI)(GGGS)3 using BamHI and XhoI restriction sites. The codons for the glycine-serine-rich linker (GGGS)3 between FkpA and e-gp41 were inserted with reverse primer for cloning of FkpA and with forward primer for cloning of e-gp41. The resulting construct was sequenced and found to encode the desired protein. E. coli harboring the expression plasmid were grown to an OD600 of 0.7, and cytosolic overexpression was induced by adding 1 mM of IPTG at a growth of temperature of 37degreesC. Four hours after induction, cells were harvested by centrifugation, and bacterial pellet was resuspended in 50 mM sodium phosphate, 5 mM imidazole and stirred at room temperature for complete lysis. After repeated centrifugation, supernatant was filtered and applied to a Ni-NTA-column, and unspecifically bound proteins were removed. Bound target protein was eluted with 50 mM sodium phosphate and collected in 4 ml fractions. After solubilization, material was transferred into physiological buffer conditions by dialysis. Refolding of the HIV-1 gp41 part of the fusion protein (ectodomain) was induced by removing guHCl from the eluted protein by dialysis against 50 mM sodium phosphate. Analysis of recombinantly produced FkpA using near UV CD showed that FkpA was essentially unstructured under the same conditions. Refolding of gp41-FkpA by dialysis resulted in a readily soluble protein complex comprising the covalently linked gp41 and FkpA protein domains. (18 pages)

L85 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
AB DERWENT ABSTRACT:

NOVELTY - Producing a soluble complex comprising a target protein which is essentially insoluble and a peptidyl-prolyl-isomerase class chaperone comprises mixing the protein and the chaperone in a buffer where both the protein and the chaperone are solubilized, and adjusting the buffer to physiological conditions where the protein-chaperone complex formed is soluble.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a soluble complex comprising a retroviral surface glycoprotein and a peptidyl prolyl isomerase chaperone, where the retroviral surface glycoprotein and the peptidyl prolyl isomerase chaperone are covalently linked; (2) a composition of reagents comprising the soluble complex; (3) a method of detecting at least one antibody to an envelope virus surface glycoprotein in a sample by contacting the sample and a composition containing a complex of the surface glycoprotein and a peptidyl prolyl isomerase chaperone, and detecting bound antibodies; (4) an immunoassay according to the double antigen bridge concept, comprising a first antigen consisting of a first chaperone-antigen complex, and a second antigen consisting of a second chaperone-antigen complex; (5) a method of eliciting an immune response by injecting into the subject a vaccine comprising a soluble retroviral surface glycoprotein-chaperone complex to elicit antibodies that bind the retroviral surface glycoprotein; and (6) a method of inhibiting virus entry into a cell by administering to a cell a soluble complex above.

BIOTECHNOLOGY - Preferred Method: The physiological buffer comprises a buffer compound in a concentration of 10-200 mM and a total salt

concentration of 20-500 mM. The protein and the peptidyl prolyl isomerase are produced recombinantly. The protein is an amyloidogenic protein, such as a retroviral surface glycoprotein, an HIV-2 gp36, or HIV-1 gp41. The peptidyl prolyl isomerase is a binding-competent fragment of the peptidyl prolyl isomerase or an FKBP chaperone selected from SlyD, FkpA and a trigger factor. The soluble complex is a retroviral surface glycoprotein-chaperone complex. The complex may be produced by solubilizing a retroviral surface glycoprotein covalently linked to a peptidyl prolyl isomerase in a buffer where the retroviral surface glycoprotein is solubilized, and adjusting the buffer to physiological conditions where the retroviral surface glycoprotein-chaperone complex is soluble. In detecting at least one antibody to an envelope virus surface glycoprotein, the sample is contacted under conditions allowing the binding of the antibodies to the surface glycoprotein and the presence of bound antibodies indicates the presence of anti-viral antibodies in the sample. In the immunoassay according to the double antigen bridge concept, the first chaperone and the second chaperone are different molecules derived from one species or from different species. The first and/or second chaperone is derived from a thermophilic bacteria. The first antigen complex comprises a solid phase binding group and the second antigen complex comprises a marker group. Inhibiting virus entry comprises inhibiting membrane fusion. The physiological buffer comprises a buffer compound in a concentration of 10-200 mM and a total salt concentration of 20-500 mM. The protein and the peptidyl prolyl isomerase are produced recombinantly. The protein is an amyloidogenic protein, preferably a retroviral surface amyloidogenic glycoprotein, an HIV-2 gp36 or an HIV-1 gp41. The peptidyl prolyl isomerase is a binding-competent fragment of the peptidyl prolyl isomerase. Preferred Complex: The retroviral surface glycoprotein and the peptidyl prolyl isomerase chaperone are covalently linked, specifically chemically coupled or recombinantly linked. The recombinant linkage comprises a peptide linker of at least 10-50 amino acids.

ACTIVITY - Immunostimulant; Anti-HIV. No biological data given.

MECHANISM OF ACTION - Vaccine. No biological data given.

USE - The method is useful for producing soluble retroviral surface glycoprotein-chaperone complex. The chaperone-antigen complex is useful in detecting antibodies to HIV in immunoassays, specifically according to the double antigen bridge concept, or as an immunogen. Compositions comprising gp41-chaperone complex and/or a gp36-chaperone complex may be used to prevent HIV entry and spread within the host organism, and for eliciting an immune response in a mammal.

ADMINISTRATION - Typical dose is 1 fg-1 mg, preferably 100 ng-50 micrograms per kg body weight. Administration can be intradermal, subcutaneous, intramuscular, intraperitoneal, inhalation, topical, by suppository, or using a transdermal patch.

EXAMPLE - The restriction site BamHI in the coding region of the mature E. coli FkpA was deleted using the QuikChange site-directed mutagenesis kit. The gene fragment encoding amino acids 535-681 from HIV-1 envelope protein was PCR amplified, and was inserted into EckFpA(DELTA~~B~~BamHI)(GGGS)<sub>3</sub> using BamHI and XhoI restriction sites. The codons for the glycine-serine-rich linker (GGGS)<sub>3</sub> between FkpA and e-gp41 were inserted with reverse primer for cloning of FkpA and with forward primer for cloning of e-gp41. The resulting construct was sequenced and found to encode the desired protein. E. coli harboring the expression plasmid were grown to an OD<sub>600</sub> of 0.7, and cytosolic overexpression was induced by adding 1 mM of IPTG at a growth of temperature of 37 degrees Centigrade. Four hours after induction, cells were harvested by centrifugation, and bacterial pellet was resuspended in 50 mM sodium phosphate, 5 mM imidazole and stirred at room temperature for complete lysis. After repeated centrifugation, supernatant was filtered and applied to a Ni-NTA-column, and unspecifically bound proteins were

removed. Bound target protein was eluted with 50 mM sodium phosphate and collected in 4 ml fractions. Unfolded gp41-FkpA polypeptide was applied on a Superdex 200 gel filtration column equilibrated with 20 mM sodium phosphate, 50 mM NaCl, 1 mM EDTA. FkpA-gp41 elutes essentially in 3 main fractions: as a high molecular associate, as an apparent hexamer species, and as an apparent trimer species. The apparent trimer fraction was concentrated and assessed for its tertiary structure in a near ultra-violet (UV) CD measurement. Gp41 displayed tertiary structure at neutral pH and was evidently solubilized by the covalently bound chaperone. The chaperone FkpA seemed to accept the native-like structured ectodomain gp41 as substrate and to solubilize this hard-to-fold protein at a neutral working pH. (36 pages)

L85 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN

AB Provided is a method for crystallization of any desired protein by recombinant expression as fusion protein with chaperonin subunit(s) for X-ray crystal structure anal. A target protein is either ligated to a chaperonin subunit via peptide bond or incorporated into the tertiary structure of the ring formed by chaperonin subunits. The target protein may be a membrane protein or nuclear hormone receptor. Expression of human cyclophilin (cyclosporin-binding protein hCyp) as fusion protein with Thermococcus chaperonin  $\alpha$  subunit and expression of GFP as fusion protein with E. coli GroEL, and X-ray crystal structure study, are described.

L85 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN

AB A method is provided for promoting the folding of a polypeptide comprising at least one Ig domain which method comprises contacting the polypeptide with a mol. chaperone and a foldase.

L85 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN

AB The chaperonin GroEL and the peptidyl-prolyl cis-trans isomerase cyclophilin are major representatives of two distinct cellular systems that help proteins to adopt their native three-dimensional structure: mol. chaperones and folding catalysts. Little is known about whether and how these proteins cooperate in protein folding. In this study, we have examined the action of GroEL and cyclophilin on a substrate protein in two distinct prolyl isomerization states. Our results indicate that: (i) GroEL binds the same substrate in different prolyl isomerization states. (ii) GroEL-ES does not promote prolyl isomerizations, but even retards isomerizations. (iii) Cyclophilin cannot promote the correct isomerization of prolyl bonds of a GroEL-bound substrate, but acts sequentially after release of the substrate from GroEL. (I.v.) A denatured substrate with all-native prolyl bonds is delayed in folding by cyclophilin due to isomerization to non-native prolyl bonds; a substrate that has proceeded in folding beyond a stage where it can be bound by GroEL is still sensitive to cyclophilin. (V) If a denatured cyclophilin-sensitive substrate is first bound to GroEL, however, productive folding to a cyclophilin-resistant form can be promoted, even without GroES. We conclude that GroEL and cyclophilin act sequentially and exert complementary functions in protein folding.

=> fil .becpat

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
45.89	46.10

FULL ESTIMATED COST

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE	TOTAL
ENTRY	SESSION
-2.40	-2.40

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FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 16:47:12 ON 09 JAN 2008

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### 3 FILES IN THE FILE LIST

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FILE 'BIOTECHDS'

24291 WO/PC  
29029 PRY<=2003  
(PRY<=2003)  
107083 PY>=2004  
(PY>=2004)

L86 2 (L40 OR L64) AND WO/PC AND PRY<=2003 AND PY>=2004

FILE 'HCAPLUS'

286232 WO/PC  
800112 PRY<=2003  
4774876 PY>=2004

L87 5 (L43 OR L67) AND WO/PC AND PRY<=2003 AND PY>=2004

FILE 'WPIDS'

507817 WO/PC  
1502382 PRY<=2003  
3288202 PY>=2004  
(PY>=2004)

L88 3 (L47 OR L71) AND WO/PC AND PRY<=2003 AND PY>=2004

TOTAL FOR ALL FILES

L89 10 (L48 OR L72) AND WO/PC AND PRY<=2003 AND PY>=2004

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PROCESSING COMPLETED FOR L89

L90 7 DUP REM L89 (3 DUPLICATES REMOVED)

=> d tot

L90 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN

TI Versatile platform for nanotechnology based on circular permutations of  
chaperonin TF55 $\beta$  from *Sulfolobus shibatae*

SO U.S. Pat. Appl. Publ., 151 pp., Cont.-in-part of U.S. Ser. No. 494,853.  
CODEN: USXCO

IN Paavola, Chad D.; Trent, Jonathan D.; Chan, Suzanne L.; Li, Yi-Fen;  
McMillan, R. Andrew; Kagawa, Hiromi

AN 2006:367306 HCAPLUS

DN 144:407655

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2006084792	A1	20060420	US 2005-194991	20050801 <--
WO 2003080796	A2	20031002	WO 2002-US35889	20021108 <--
WO 2003080796	A9	20040429		
WO 2003080796	A3	20051222		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2005130258	A1	20050616	US 2004-494853	20040506 <--

L90 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

TI Fusion protein useful for preparing target protein such as  
immunoglobulin, has molecular chaperone connected to terminal of  
intercalated protein having activity of cleaving peptide bond, and target  
protein;

isolation of a recombinant fusion protein having intein and green fluorescent protein useful for production of immunoglobulin

AU TOGI A; FURUTANI M  
AN 2005-00070 BIOTECHDS  
PI WO 2004096860 11 Nov 2004

L90 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
TI Novel immunogen comprising fused protein of full-length or part of antigen (serotonin 5 HT1aR) and folding factor (peptidylprolyl isomerase) or its subunit bonded together through peptide bonds, useful for inducing immune response;

vector-mediated immunogen gene transfer and expression in host cell for recombinant vaccine

AU IZUMOTO Y; HATA J; IDENO A; FURUTANI M  
AN 2004-25618 BIOTECHDS  
PI WO 2004092221 28 Oct 2004

L90 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN DUPLICATE 3

TI Production of recombinant antibodies as chaperonin fusion protein

SO Jpn. Kokai Tokkyo Koho, 23 pp.

CODEN: JKXXAF

IN Ideno, Akira; Hata, Junichi; Togi, Akiko; Furuya, Masahiro

AN 2004:216907 HCAPLUS

DN 140:265615

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2004081199	A	20040318	JP 2003-124351	20030428 <--
WO 2004097018	A1	20041111	WO 2004-JP1839	20040218 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

L90 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN

TI Chaperonin-target protein complex, method of producing the same, method of stabilizing target protein, method of immobilizing target protein, method of analyzing the structure of target protein, sustained-release preparation and method of producing antibody against target protein

SO PCT Int. Appl., 48 pp.

CODEN: PIXXD2

IN Ideno, Akira; Hata, Jun-ichi; Togi, Akiko; Furutani, Masahiro

AN 2004:965292 HCAPLUS

DN 141:428008

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004096859	A1	20041111	WO 2004-JP6189	20040428 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

AU 2004234282 A1 20041111 AU 2004-234282 20040428 <--

CA 2522263 A1 20041111 CA 2004-2522263 20040428 <--  
 EP 1619208 A1 20060125 EP 2004-730054 20040428 <--  
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, HR  
 US 2007059794 A1 20070315 US 2005-554747 20051028 <--

L90 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN

TI Soluble complexes of amyloid  $\beta$  (A $\beta$ ) target proteins and  
 peptidyl prolyl isomerase (Ppplase)

chaperones, their preparation and use in diagnosis of Alzheimer's disease and  
 as immunogens

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

IN Faatz, Elke; Scholz, Christian; Schaarschmidt, Peter

AN 2004:546522 HCAPLUS

DN 141:102770

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004056855	A1	20040708	WO 2002-EP14631	20021220 <--
WO 2004056855	A9	20050714		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,				
UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,				
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,				
FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ,				
CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2510717	A1	20040708	CA 2002-2510717	20021220 <--
AU 2002363891	A1	20040714	AU 2002-363891	20021220 <--
EP 1578793	A1	20050928	EP 2002-798355	20021220 <--
EP 1578793	B1	20071003		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
JP 2006522583	T	20061005	JP 2004-561108	20021220 <--
AT 374784	T	20071015	AT 2002-798355	20021220 <--
US 2005112720	A1	20050526	US 2003-443654	20030522 <--
US 7094884	B2	20060822		

L90 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN

TI Expression vector containing chaperonin PPIase for improvement  
 of the expression efficiency for foreign proteins

SO PCT Int. Appl., 73 pp.

CODEN: PIXXD2

IN Ideno, Akira; Maruyama, Tadashi; Furutani, Masahiro

AN 2004:3039 HCAPLUS

DN 140:72133

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004001041	A1	20031231	WO 2003-JP8020	20030625 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
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GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,				
PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR,				
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,				
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,				
FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,				
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2490384	A1	20031231	CA 2003-2490384	20030625 <--
AU 2003243969	A1	20040106	AU 2003-243969	20030625 <--
EP 1516928	A1	20050323	EP 2003-733573	20030625 <--



R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK  
US 2005130259 A1 20050616 US 2004-511098 20041014 <--

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L90 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN

AB The present invention provides chaperonin polypeptides which are modified to include N-terminal and C-terminal ends that are relocated from the central pore region to various different positions in the polypeptide which are located on the exterior of the folded modified chaperonin variant. In the modified chaperonin, the naturally-occurring N-terminal and C-terminal ends are joined together directly or with an intervening linker peptide sequence. The relocated N-terminal or C-terminal ends can be covalently joined to, or bound with another mol. such as a nucleic acid mol., a lipid, a carbohydrate, a second polypeptide, or a nanoparticle. The modified chaperonin variants can assemble into double-ringed chaperonin structures. Further, the chaperonin structures can organize into higher order structures such as nanofilaments or nanoarrays which can be used to produce nanodevices and nanocoatings. In particular, the invention provides modified variants of *Sulfolobus shibatae* chaperonin TF55 $\beta$ . The sequences of the *S. shibatae* chaperonin TF55 $\beta$  modified variants are provided.

L90 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

AB DERWENT ABSTRACT:

NOVELTY - A fusion protein (I) comprises a molecular chaperone or its subunit connected to a terminal of intercalated protein which has an activity of cleaving a peptide bond, and a target protein connected to other terminal by a peptide bond.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated gene (II) encoding (I); (2) an isolated gene (III) encoding a partial sequence of intein protein; (3) an expression vector (IV) comprising (II) or (III); (4) a transformed host comprising (IV); and (5) preparing a target protein, involves: (a) connecting a molecular chaperone or its subunit to a terminal of partial sequence of intein protein by a peptide bond, producing first fusion protein which connected a target protein first precursor to the other terminal by a peptide bond, connecting a molecular chaperone or its subunit to a terminal of the remaining partial sequence of intein protein by a peptide bond, producing a second fusion protein which connected the target protein second precursor to the other terminal by a peptide bond, cutting a target protein first precursor from the first fusion protein obtained in the above process by the cleavage action of the partial sequence of intein protein and the remaining partial sequence of the intein protein, further cutting the target protein second precursor from the second fusion protein obtained by the above mentioned process, and assembling the target protein first precursor and target protein second precursor obtained by the above process, by the assembly function of partial sequence of intein protein and the remaining partial sequence of the intein protein; or (b) connecting a partial sequence of intein protein to a terminal of molecular chaperone, and connecting the remaining partial sequence of the intein protein to the other terminal by a peptide bond, further connecting a target protein first precursor to the partial sequence of intein protein by a peptide bond, and from a fusion protein having the target protein first precursor and the target protein second precursor connected by a peptide bond, cutting target protein first precursor and target protein second precursor by the cleavage action of the partial sequence of intein protein and remaining partial sequence of intein protein, and further assembling the target protein first precursor and target protein second precursor by the assembly function of the partial sequence of intein protein and remaining partial sequence of intein protein.

BIOTECHNOLOGY - Preferred Protein: In (I), the molecular chaperone

is a chaperonin containing a number of chaperonin subunits. The intercalated protein having peptide bond cleaving activity is connected to an individual chaperonin subunit, or a chaperonin subunit connecting material, where the 2-10 chaperonin subunits connected the chaperonin subunit connecting material in series through a peptide bond. The intercalated protein having a peptide bond cleaving activity is connected to the region of one or more of an individual chaperonin subunit or individual N-terminal of chaperonin subunit connecting material; the C-terminal of an individual chaperonin subunit or an individual chaperonin subunit connecting material; or the connecting portion of the chaperonin subunits of a chaperonin subunit connecting material. The ratio of the number of chaperonin subunit and target protein is 1:1-10:1. The molecular chaperone is peptidyl prolyl cis-trans isomerase, and is derived from bacteria, archaebacterium or eukaryote. The intercalated protein having peptide bond cleaving activity is connected to N-terminal and/or the C-terminal of peptidyl prolyl cis-trans isomerase. The intercalated protein having a peptide bond cleaving activity is intein or a partial sequence of intein, where intein cleaves N-terminal and does not cleave a C-terminal, or cleaves C-terminal and does not cleave N-terminal. The partial sequence of intein consists of 20-120 amino acids of C-terminal of intein. The intein is derived from *Synechocystis* sp., *Mycobacterium xenopi*, *Saccharomyces cerevisiae* or *Halobacterium* sp.. The partial sequence of intein has a fully defined sequence of 154 amino acids (S1) as given in the specification or (S1) in which one or more amino acids deleted, substituted, added or inserted, where the protein has peptide cleaving activity. The partial sequence of intein has an amino acid sequence, exhibiting at least 50% or more homology with (S1), where the protein has peptide cleaving activity. The intercalated protein having peptide cleaving activity is protease.

USE - (I) is useful for preparing a target protein, which involves: (a) cutting out the target protein from (I) by the action of the intercalated protein having a peptide cleaving activity; or (b) preparing (I), inducing peptide cleaving activity of the intercalated protein contained in (I) prepared in the above process, cleaving a portion of (I) by the action of the intercalated protein whose peptide cleaving activity is induced in the above process, and cutting out and separating the target protein from (I). The peptide cleaving activity is induced by exposing (I) to a temperature of 20-37degreesC, pH of 6-8, and by adding thiol. The method involves producing an expression vector having a gene that encodes (I), introducing the expression vector obtained by above process into a host and expressing (I), and cutting out the target protein from (I) expressed in the above process. The method involves integrating a gene encoding fusion protein in two types of different plasmids that can be co-existed and expressed within the same host, and producing two types of expression vectors, introducing the two types of expression vectors obtained in the above process into the same host and expressing (I), and cutting out the target protein by the action of intercalated protein having peptide bond cleaving activity from (I). The method involves integrating a gene that encodes (I) in one side of two types of different plasmids that can be co-existed and express within the same host, integrating a gene that encodes only the molecular chaperone on the other side, and producing two types of expression vectors; introducing the two types of expression vectors obtained by the above process into the same host; expressing (I) and the molecular chaperone; and cutting out the target protein by the action of intercalated protein having peptide cleaving activity, from (I) obtained by the above process. The host is bacteria, yeast, animal cell, plant cell, insect cell, an animal, plant or an insect. The method involves making (I) express within the host as mentioned above. The method is carried out with a non-cell translation system. The molecular chaperone is chaperonin. The 5-10 chaperonin subunits are gathered in ring shape to form a chaperonin ring, where the target protein is accommodated within the chaperonin ring (all claimed). (I) is useful in preparing immunoglobulin.

ADVANTAGE - (I) enables to prepare a target protein, efficiently.

(I) is expressed more easily by gene modification technology. The intercalated protein having peptide bond cleaving activity enables to cut the target protein safely and conveniently.

EXAMPLE - Expression of fusion protein was carried out as follows. A plasmid pETTPPIaseI having T7 promoter, peptidyl prolyl cis-trans isomerase gene (TPPIase), *Synechocystis* sp. intein gene (SspI) and His gene was prepared. The gene encoding green fluorescent protein (GFP) was introduced between SpeI site and HpaI site. Thus, the expression vector pETTPPIase-GFP capable of synthesizing fusion protein of SspI and GFP was built. The obtained expression vector was introduced into *Escherichia coli* BL21 (DE3) strain. The transformed organism was cultivated in YT culture medium containing carbenicillin at 25degreesC for 24 hours. Thus, a fusion protein comprising TPPIase, SspI and GFP, in the culture was obtained. (76 pages)

L90 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
AB DERWENT ABSTRACT:

NOVELTY - An immunogen (I) for inducing an immune response against a desired antigen protein, comprises a fused protein in which the full-length or a part of a desired antigen protein and a folding factor or its subunit are bonded together through one or more peptide bonds, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) producing (I), involves translating a fusion gene that encodes a full-length or a part of a desired antigen protein and a folding factor or its subunit; and (2) composition (II) for immunological use, formed by mixing (I) and an adjuvant.

BIOTECHNOLOGY - Preferred Immunogen: In (I), the folding factor is chaperonine, which comprises number of chaperonine subunits. One or all parts of the chaperonine subunits are mutually connected by peptide bonds. The desired antigen protein is connected to the N- or C-terminal of the chaperonine subunits. The antigen protein is connected among the chaperonine subunits. (I) has a protease cleavage site between the chaperonine subunits and the antigenic protein. The chaperonine subunits are derived from bacteria, preferably archaeobacterium or from eukaryotes. The antigen protein is contained inside the chaperonine ring formed by the chaperonine subunits. The chaperonine ring comprises 5-10 chaperonine subunits. The folding factor includes chaperonine and PPIase. The PPIase is derived from *Escherichia coli* or archaeobacterium. The antigenic protein is serotonin 5 HT1aR. The fusion protein comprises full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR.

ACTIVITY - Immunostimulant.

MECHANISM OF ACTION - None given.

USE - (I) or (II) is useful for producing an antibody specific to the antigen protein, which involves immunizing an animal (except human) using (I) or (II) and extracting the antibody from the animal (claimed).

ADVANTAGE - (I) prevents the antigen protein from quick degradation in the blood of an animal. (I) provides effective immune response against a desired antigenic protein and enables reliable production of an antibody against the desired antigenic protein.

EXAMPLE - Immunogen comprising *Escherichia coli* derived chaperonine GroEL and an antigenic protein was produced as follows. Vector capable of expressing the fusion protein of GroEL and antigenic protein was assemble. The antigenic protein is full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR. The vector was introduced into a suitable host and the fusion protein comprising 7 subunits of GroEL and recombinant serotonin 5 HT1aR receptor was produced. The stereostructure of the obtained fusion protein was examined. The structure analysis showed that the chaperonine subunits formed a ring structure inside which the antigenic protein was contained. The serotonin 5 HT1aR receptor was protected by GroEL. The immunogenicity of the fusion protein (immunogen) was evaluated. The fusion protein was admixed with incomplete Freund's

adjuvant and a composition for immunological use was obtained. The composition was administered (subcutaneously) to a rabbit and antibody titer against serotonin 5 HT1aR receptor was measured. The results showed that the immunogen was efficient in inducing immune response against serotonin 5 HT1aR receptor (antigenic protein). (59 pages)

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AB Provided is a method of antibody production by expressing heavy chain and/or light chain as fusion protein with chaperonin and reconstituting after protease cleavage from the fusion partner. 2-10 Chaperonin subunits are linked via peptide bond and antibody heavy chain and/or light chain are linked to its N-terminal, C-terminal, or linkage between subunits. A preferred form is where 2 chaperonin rings comprising 5-10 chaperonin subunits form a noncovalent two-layer structure via the ring surface and antibody heavy chain and/or light chain are enclosed within. Humanized antibodies or chimeric antibodies may be produced. Fusion proteins of human anti-hepatitis B surface antigen (HBs) antibody heavy chain and of light chain with Thermococcus KS-1 chaperonin  $\beta$  subunit were produced in E. coli. Binding ability of the antibody reconstituted from heavy chain and light chain was higher than that of the heavy chain alone.

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AB It is intended to provide a complex of chaperonin and a target protein by which the target protein can be handled more easily, and a method of producing the same; and a method of stabilizing the target protein, a method of immobilizing the target protein, a method of analyzing the structure of the target protein, a sustained-release agent and a method of producing an antibody against the target protein, each using the chaperonin-target protein complex. The above-described chaperonin-target protein complex contains a fused protein in which an affinity tag is attached to chaperonin subunits via a peptide bond, and a target protein to which the affinity tag shows a specific affinity. Owing to the specific affinity, the target protein is bonded to the affinity tag, thus forming a chaperonin ring structure consisting of a plural number of chaperonin subunits. Using to the chaperonin-target protein complex, the target protein can be stabilized and surely immobilized on a support without causing any change in its stereostructure. Thus, IgG was incubated with a chaperonin  $\beta$ -subunit-protein A fusion protein produced in E. coli to obtain a chaperonin-IgG complex.

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AB The disclosed invention relates to the production of a soluble A $\beta$ -chaperone complex and wherein the chaperone is a member of the Ppplase family and the advantageous use of such chaperone-A $\beta$  complex, especially in the detection of A $\beta$  in an immunoassay, as well as its use as an immunogen. In a presented experiment an expression vector was constructed comprising E. coli-derived SlyD chaperone as the fusion partner and A $\beta$ (1-42) as target protein. The methods described facilitate the convenient recombinant production of an A $\beta$  in a soluble form and in high amts. (yield >20 mg fusion protein/g wet weight of E. coli cell mass).

=> log y

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
43.44	89.54

FULL ESTIMATED COST

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE	TOTAL
ENTRY	SESSION
-3.20	-5.60

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